Key Factors in “Katsuobushi” (Dried Bonito) Aroma Formation

Hiroyuki Imai, Tetsuo Aishima* and Akio Nobuhara

Central Research Laboratories, Kikkoman Corp.,
399 Noda, Noda-shi, Chiba 278, Japan
*Noda Institute for Scientific Research,
399 Noda, Noda-shi, Chiba 278, Japan

Received May 29, 1981

The necessary factors for making the characteristic aroma of Katsuobushi were investigated. The aromas, obtained from various heat treatments of several materials, were compared with the aroma of Katsuobushi using a sensory test, pattern similarity analysis of GC profiles and GC-MS data. The oil and meat of bonito and the smoking process were considered significant among several factors in the Katsuobushi manufacturing process. However extracted residue and oil of Katsuobushi, i.e. the protein-rich and oil fractions, contained only a trace of Katsuobushi aroma, the mixture of these two materials had a Katsuobushi-like aroma after heating with smoke oil as a substitute for the smoking process. The similarity in the aroma qualities between the obtained Katsuobushi-like and genuine Katsuobushi aromas was confirmed by comparing the aroma components and GC profiles of both aromas.

Aroma research on Katsuobushi, i.e. dried bonito (Katsuwonus pelamis), with gas chromatography increased the number of identified compounds to one hundred.1~6) But no one compound can characterize the preferable Katsuobushi aroma.

According to Nursten,7) the aroma of fifteen foods consisting of fresh vegetables and fruits are ascribed to only one “character impact” compound. The aromas of most processed foods are considered as no more but the integrated effects of many aroma compounds contained in them.8) Therefore, the statistical approach has been applied to the aroma research of processed foods since 19689) and has revealed close relationships between aroma quality and their GC profiles.10~12)

The Katsuobushi manufacturing process is time-consuming and consists of several steps such as boiling, sun-drying, smoking and molding. Consequently, the aroma components in Katsuobushi are formed through these steps. Therefore, the origin of Katsuobushi aroma can be separated into the heat reaction of the oil- and water-soluble components contained in bonito and their mutual interaction during the heat treatment. The other two processes, i.e. smoking and molding, were also considered significant for formation of the Katsuobushi aroma.

The purpose of our investigation was to discover the key factors and conditions for the aroma formation of Katsuobushi by selecting factors deduced from the manufacturing process.

Firstly the effect of molding on the aroma of Katsuobushi was examined by comparing the aroma of molded Katsuobushi with that of unmolded Katsuobushi on GC and GC-MS data. However, both the aroma of molded Katsuobushi and that of unmolded Katsuobushi was peculiar to genuine Katsuobushi. Then, other major factors for the aroma formation of Katsuobushi were investigated by selecting materials in order to make model systems. The model system, consisting of fish oil, a protein-rich fraction and smoke oil, were heated under different conditions. Then the aroma of the resulting reactants were organoleptically compared with that of genuine Katsuobushi. The aromas extracted from the reactants were analyzed by GC and GC-MS, and their GC patterns compared by a simple pattern similarity analysis.
method. The successful applications of the pattern similarity analysis for amino acids in foods\textsuperscript{13} and Katsuobushi aroma\textsuperscript{14} have already been reported.

**MATERIALS AND METHODS**

*Materials and sample preparation for GC analysis.* Ordinary molded Katsuobushi and unmolded Katsuobushi produced in Yaizu were pulverized. 300 g of the pulverized katsuobushi was extracted with 900 ml of 80\% aqueous ethanol at 80°C for one hour and the extraction repeated five times until the aroma components were completely eliminated from the Katsuobushi powder.

The resulting extract was concentrated up to 450 ml by a rotary evaporator at 40°C. The concentrate was distilled under a reduced pressure, 15 Torr, and at 45°C by blowing nitrogen gas into the concentrate through a glass capillary. The condensed distillates, cooled with ice-water and dry ice-ethanol traps, were then combined. The mixture was treated with dichloromethane for GC analysis by using n-tridecane as the internal standard. The dichloromethane extract was concentrated under a reduced pressure at 30 Torr and one portion of the resulting condensate was injected into GC.\textsuperscript{12}

*Formation of Katsuobushi aroma by heat treatments of model systems.* The following three (I ~ III) fractions were selected for model systems in order to investigate formation of the Katsuobushi aroma.

(I) Protein-rich fraction including extracted residue of unmolded Katsuobushi and soybean protein purchased in the market.

(II) Oil fraction including Katsuobushi oil isolated from the aqueous ethanol extract of Katsuobushi, bonito oil, tuna oil, cod oil and sardine oil. The four fish oils were prepared from boiled extracts of each fish.

(III) Smoke oil (Banyu Eiyo Co., Ltd.) was used as a substitute for the smoking process.

The combinations and their mixing ratios were sequentially changed. A mixture in a petri dish was put in an oven for heat treatment. The conditions of heat treatment are shown in Fig. 1. Firstly, the mixture was heated for one hour at 100°C. Then, while smoke oil and water were added at intervals of one hour, the mixture was heated for four hours. When protein-rich fraction was removed in the mixture, filter paper was used as support for oil, smoke oil and water during the heat treatment. The aromas in the treated mixtures were extracted and concentrated using the same method as for Katsuobushi aroma.

*GC analysis.* A Hitachi K-53 gas chromatograph equipped with FID was used for GC analysis and the analytical conditions were as follows. Column: glass, packed with 20\% PEG20M on Chromosorb W AW-DMCS, 3 m length and 3 mm i.d. Oven temperature was increased from 50°C to 230°C at the rate of 3°C/min. The area of each peak on the gas chromatogram was measured by a Takedariken TR-2213 integrator.

*GC-MS analysis.* The aroma concentrates were analyzed in a combined Hitachi gas chromatograph 063-RMU-7M mass spectrometer. The gas chromatographic separation was performed with a 30 m glass capillary column coated with FFAP. Mass spectra were recorded at 70 eV. The temperatures of the separator and ion source were 260°C and 220°C, respectively. The compounds in the aroma concentrates were identified by comparing their mass spectra with reference spectra\textsuperscript{15} and their GC retention times with authentic compounds.

*Pattern similarity analysis.*\textsuperscript{13,14} Pattern similarity $S(A, B)$ and radian distance $R(A, B)$ were calculated from eqs. I and II, respectively.

<table>
<thead>
<tr>
<th>Protein-rich fraction (extracted residue of Katsuobushi, soybean protein)</th>
<th>70 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil fraction (Katsuobushi oil, bonito oil, tuna oil, cod oil, sardine oil)</td>
<td>9 g</td>
</tr>
<tr>
<td>Smoke oil</td>
<td>0.9 g</td>
</tr>
<tr>
<td>Water</td>
<td>20 ml</td>
</tr>
</tbody>
</table>

Heating (100°C, 1 hr)

Heating (100°C, 1 hr x 4)

Katsuobushi-like aroma

![Fig. 1. Process of Formation of Katsuobushi-like Aroma.](image-url)
A GC-MS analysis of the aroma concentrates of molded and unmolded Katsuobushi showed that the same components were contained in both aromas as listed in Table I. The forty-eight compounds were commonly identified in the aroma concentrates of molded and unmolded Katsuobushi. Seventeen of them were not previously reported as being present in Katsuobushi aroma but 11 compounds among the newly identified 17 had been reported as constituents in the aroma of smoke oil.\textsuperscript{17}~\textsuperscript{20} GC profiles of the aroma concentrates of molded and unmolded Katsuobushi looked alike as shown in Fig. 2. According to the calculation from the 18 peaks marked in Table I, the pattern similarity and radian distance between the two gas chromatograms were 0.988 and 0.156, respectively. The selection of these peaks was performed on the basis of previous information on Katsuobushi aroma.\textsuperscript{2}~\textsuperscript{5}~\textsuperscript{14} The high similarity indicated that the patterns of the two chromatograms were almost the same on the basis of the significant components.

\begin{table}[h]
\centering
\caption{Aroma Components of Unmolded and Molded Katsuobushi}
\begin{tabular}{|c|c|c|c|}
\hline
Peak number & Compound & Percentage composition & Evidence \\
\hline
1 & 1-Penten-3-ol & 0.44 & MS, GC \\
2 & 1-Pentenol & — & MS, GC \\
3 & Methylpyrazine\textsuperscript{17} & — & MS, GC \\
4 & 3-Hydroxy-2-butanone\textsuperscript{18} & 0.06 & MS, GC \\
 & Cyclopentanol\textsuperscript{17} & 0.05 & MS, GC \\
5 & 2,5-Dimethylpyrazine & 0.13 & MS, GC \\
6 & 2,6-Dimethylpyrazine & 0.58 & MS, GC \\
7 & Unknown (MS m/z: 45, 75, 43) & 10.58 & MS, GC \\
8 & 2,3-Dimethylpyrazine\textsuperscript{17} & — & MS \\
 & 2-Cyclopenten-1-one\textsuperscript{17,19} & — & MS \\
9 & 2-Methyl-2-cyclopenten-1-one\textsuperscript{18} & — & MS \\
10 & 2-Methyl-5-ethylpyrazine & — & MS \\
 & 3-Methyl-5-ethylpyrazine & — & MS \\
11* & Trimethylpyrazine & 0.96 & MS, GC \\
12 & 1-Octen-3-ol & 1.15 & MS, GC \\
13 & Tetramethylpyrazine\textsuperscript{1} & 0.87 & MS, GC \\
 & Acetic acid & 1.54 & MS, GC \\
\hline
\end{tabular}
\end{table}
<table>
<thead>
<tr>
<th>Peak number</th>
<th>Compound</th>
<th>Percentage composition</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Unmolded</td>
<td>Molded</td>
</tr>
<tr>
<td>14 ~ 17</td>
<td>Unknown</td>
<td>0.02</td>
<td>0.11</td>
</tr>
<tr>
<td>18</td>
<td>Propionic acid</td>
<td>0.08</td>
<td>0.14</td>
</tr>
<tr>
<td>19</td>
<td>Unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>2-Methylpropanoic acid</td>
<td>0.17</td>
<td>0.15</td>
</tr>
<tr>
<td>21</td>
<td>4-Pentanolide*</td>
<td>3.95</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td>Trimethyl-2-cyclopenten-1-one†</td>
<td>6.37</td>
<td>6.43</td>
</tr>
<tr>
<td>22</td>
<td>4-Butanolide†</td>
<td>3.74</td>
<td>5.30</td>
</tr>
<tr>
<td>23</td>
<td>Pentanolide*</td>
<td>6.37</td>
<td>6.43</td>
</tr>
<tr>
<td>24</td>
<td>Hexanal</td>
<td>0.01</td>
<td>0.39</td>
</tr>
<tr>
<td>25</td>
<td>Unknown</td>
<td>2.44</td>
<td>0.47</td>
</tr>
<tr>
<td>26</td>
<td>Unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>2-Methylbutanoic acid</td>
<td>11.23</td>
<td>1.63</td>
</tr>
<tr>
<td>28</td>
<td>Unknown</td>
<td>0.45</td>
<td>0.12</td>
</tr>
<tr>
<td>29</td>
<td>3-Methyl-2(5H)-furanone†</td>
<td>0.28</td>
<td>0.57</td>
</tr>
<tr>
<td>30</td>
<td>Unknown</td>
<td>3.16</td>
<td>4.17</td>
</tr>
<tr>
<td>31*</td>
<td>Phenol</td>
<td>14.64</td>
<td>15.31</td>
</tr>
<tr>
<td></td>
<td>2-Methylphenol</td>
<td>3.16</td>
<td>4.17</td>
</tr>
<tr>
<td></td>
<td>1-Indanone†</td>
<td>6.05</td>
<td>6.37</td>
</tr>
<tr>
<td>32*</td>
<td>2,3-Dimethylphenol</td>
<td>0.62</td>
<td>1.89</td>
</tr>
<tr>
<td>33*</td>
<td>2,4-Dimethylphenol</td>
<td>2.68</td>
<td>5.80</td>
</tr>
<tr>
<td></td>
<td>3-Methylphenol</td>
<td>2.68</td>
<td>5.80</td>
</tr>
<tr>
<td>34*</td>
<td>3,5-Dimethylphenol</td>
<td>0.69</td>
<td>2.73</td>
</tr>
<tr>
<td>35</td>
<td>4-Ethyl-2-methoxyphenol</td>
<td>0.05</td>
<td>0.85</td>
</tr>
<tr>
<td>36</td>
<td>3,4-Dimethylphenol</td>
<td>8.45</td>
<td>8.71</td>
</tr>
<tr>
<td>37*</td>
<td>3-Methylphenol</td>
<td>3.06</td>
<td>4.14</td>
</tr>
<tr>
<td></td>
<td>4-Methylphenol</td>
<td>1.71</td>
<td>1.36</td>
</tr>
</tbody>
</table>

* Used for calculation of $S(A, B)$ and $R(A, B)$.
† Newly found in Katsuobushi aroma.
Aroma formation of Katsuobushi by heat treatments of model systems

The Katsuobushi oil, extracted residue of Katsuobushi and smoke oil were selected as significant factors for formation of the Katsuobushi aroma.

Prior to heat treatment, the aroma in each material was extracted and analyzed by GC as shown in Fig. 3. The oil and extracted residue of Katsuobushi contained aroma components in very small quantities and these GC patterns were different from the pattern of unmolded Katsuobushi as shown in Table II. The gas chromatogram of the extracted residue of Katsuobushi shows the complete elimination of aroma with aqueous ethanol.

However, the total quantity of aroma components in smoke oil was larger than that in the unmolded Katsuobushi, but the GC pattern of smoke oil was not similar to that of Katsuobushi.

The three materials were mixed and then the heat treatment was performed under the conditions shown in Fig. 1. The aroma of the heat reaction was almost similar to that of the unmolded Katsuobushi, and the GC profile is shown in Fig. 4. The pattern similarity and radian distance between the GC profiles of the unmolded Katsuobushi and the aromas formed were calculated as shown in Table III. Every compound found in the Katsuobushi aroma listed in Table I was also identified in the aroma of heat reaction of the three materials by GC-MS analysis.

\[ \text{Whole aroma quantity} = \frac{\text{sum of area of whole peak (} \mu \text{V)}}{\text{peak area of } n\text{-tridecane (} \mu \text{V)}} \]
Fig. 3. Gas Chromatograms of Aromas in Extracted Residue of Katsuobushi (A), Katsuobushi Oil (B) and Smoke Oil (C).
* Whole aroma quantity. See legend for Fig. 2.

Heat reaction of two materials
Three different combinations were made by selecting two materials from Katsuobushi oil, extracted residue of Katsuobushi and smoke oil. Each of the mixtures consisting of two materials were subjected to the heat treatment
TABLE II. PATTERN SIMILARITY AMONG GC PROFILES OF AROMAS IN THREE MATERIALS FOR FORMATION OF KATSUOBUSHI AROMA

<table>
<thead>
<tr>
<th></th>
<th>Katsuobushi</th>
<th>Residue*</th>
<th>Katsuobushi oil</th>
<th>Smoke oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>S(A, B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Katsuobushi</td>
<td>—</td>
<td>0.592</td>
<td>0.885</td>
<td>0.794</td>
</tr>
<tr>
<td>Residue*</td>
<td>0.937</td>
<td>—</td>
<td>0.630</td>
<td>0.583</td>
</tr>
<tr>
<td>Katsuobushi oil</td>
<td>0.485</td>
<td>0.889</td>
<td>—</td>
<td>0.783</td>
</tr>
<tr>
<td>Smoke oil</td>
<td>0.654</td>
<td>0.948</td>
<td>0.671</td>
<td>—</td>
</tr>
</tbody>
</table>

* Extracted residue of Katsuobushi.

FIG. 4. Gas Chromatogram of Katsuobushi-like Aroma Formed by Heat Treatment of Extracted Residue of Katsuobushi, Katsuobushi Oil and Smoke Oil.
* Whole aroma quantity. See legend for Fig. 2.

TABLE III. PATTERN SIMILARITY BETWEEN GC PROFILES OF AROMA FORMED BY HEAT TREATMENTS OF THREE MATERIALS AND KATSUOBUSHI AROMA

<table>
<thead>
<tr>
<th></th>
<th>Katsuobushi</th>
<th>Residue*/Katsuobushi oil/smoke oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>S(A, B)</td>
<td>1.0</td>
<td>0.909</td>
</tr>
<tr>
<td>R(A, B)</td>
<td>0.0</td>
<td>0.430</td>
</tr>
</tbody>
</table>

* Extracted residue of Katsuobushi.

None of the three combinations formed a Katsuobushi-like aroma. The GC profiles of the aromas of these reactions are shown in Fig. 5. The matrix of pattern similarity and radian distance among them was calculated as shown in Table IV. The closest relationship was found between Katsuobushi oil/smoke oil and Katsuobushi residue/smoke oil, but the highest pattern similarity was only derived from the effect of the aroma components in smoke oil.

The greatest pattern similarity between Katsuobushi aroma and the three reactants was found in Katsuobushi residue/smoke oil but it was only 0.838.

shown in Fig. 1 in order to discover the indispensable combination of factors for forming the aroma of Katsuobushi.
Fig. 5. Gas Chromatograms of Aromas Formed by Heat Treatments of Two Materials. Combinations of Two Materials were as Follows: A, Extracted Residue of Katsuobushi and Katsuobushi Oil; B, Extracted Residue of Katsuobushi and Smoke Oil; C, Katsuobushi Oil and Smoke Oil.
* Whole aroma quantity. See legend for Fig. 2.

Heat treatment of three materials substituting one with another material
The Katsuobushi oil and the extracted residue of Katsuobushi were selectively replaced by other materials as shown in Table V. This was tried to investigate the possibility of substitutes for Katsuobushi oil and the extracted residue of Katsuobushi as basic materials for
formulation of the Katsuobushi aroma.

The highest similarity between the GC patterns of Katsuobushi aroma and the reactants was shown by the aroma in the mixture consisting of extracted residue of Katsuobushi, bonito oil and smoke oil. But the value was only 0.835.

Aromas derived from combinations of the other three materials were not similar to that of unmolded Katsuobushi not only in sensory characteristics but also in their GC profiles as shown in Table V. The lowest similarity between the aroma derived from soybean protein and Katsuobushi aroma indicated that significant precursors for the aroma were contained in the extracted residue of Katsuobushi.

These heat reactions of arbitrary combinations of various materials clearly showed that the three materials, i.e. Katsuobushi oil, extracted residue of Katsuobushi and smoke oil, were indispensible to form the Katsuobushi-like aroma.

However although the precursors for Katsuobushi aroma and the reaction mechanism during heat treatment have not been elucidated yet, these phenomena suggest an interesting reaction between components both in Katsuobushi oil and extracted residue of Katsuobushi, aided by phenolic compounds in the smoke oil.

Acknowledgment. The authors wish to thank Mr. N. Nunomura and Dr. M. Sasaki for GC-MS analysis.

REFERENCES
2) K. Kim, T. Yamanishi, Y. Nakatani and T. Matsui, Nippon Nogeikagaku Kaishi, 45, 328 (1971).
428

H. Imai, T. Aishima and A. Nobuhara


10) R. C. Lindsay, Objective Measurement of Flavor Quality of Beer, ACS Symposium Series No. 51, 89 (1977).


